Evaluation of smell and miRNA biomarkers in Alzheimer's Disease (AD)

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ABSTRACT

Introduction: Recent studies identified dysregulated miRNAs in cerebral spinal fluid and serum from AD patients (Burgos et al, 2014). Other studies have identified miRNA profiles in disease pathologies from saliva (Bonne and Wong, 2012; Park et al, 2009), suggesting that this may also be possible in AD. This could lead to a non-invasive procedure that correlates biomarkers directly to AD for a quantitative diagnosis of this disease.

Objective: To evaluate the potential for using a combination of SLUMS testing, smell testing and miRNA isolated from saliva as biomarkers in the diagnosis of AD.

Methods: Subjects first were evaluated using a St. Louis University Mental Status (SLUMS) exam, followed by collection of saliva and administration of the University of Pennsylvania Smell Identification test. Saliva was treated with protectant for stabilization of miRNA that was subsequently extracted from AD and control subjects after being treated with a Spike In-Control, which was used to determine the reliability of the extraction. Analysis of the miRNA samples was performed to determine recovery of Spike-In Control by RT-PCR using a standard curve. A Qiagen analytical program was used to analyze data from inflammatory response and autoimmunity miRNA arrays to determine expression results from AD subjects in comparison to controls.

Results: Individuals with low SLUMS scores appeared to also have low smell test scores. miRNA analysis for inflammation between age and gender matched individuals showed a significant difference in the fold change between AD and controls. miRNA analysis is ongoing to determine if there is a correlation between fold regulation of miRNA with SLUMS and Smell Tests.

Conclusion: Evaluation of saliva for miRNA was successful. Preliminary data indicate that there is correlation between the SLUMS exam and smell test, as well as, a change in miRNA regulation between AD when compared to it's control group. Analysis of specific miRNA changes is ongoing.

INTRODUCTION

Alzheimer's Disease (AD) incidence increases with age and is considered to be one of the most common causes of dementia by effecting 5.4 million people over the age of 65 in the US (Alzheimer's & Dementia, 2012; Balin and Hudson, 2014). The neuropathology in, both early-onset and late-onset, AD is characterized by modified tau proteins forming neurofibrillary tangles (NFT) and neurophil threads (NT), as well as, deposits of β-amyloid peptide (Aβ) that lead to the formation of neuritic senile plaques (NSP). However the biological process in late-onset AD leading to this pathology is still unknown (Balin and Hudson, 2014; Lippa et al, 1996). In addition, there are many discrepancies when diagnosing late-onset AD (oppose to other forms of dementia), which is also attributed to by the limited availability of both invasive and non-invasive procedures to confirm the neuropathology that is known to be a signature of AD (Burgos et al, 2014; Jin et al 2013).

A small, endogenous non-coding RNA that is known to regulate post-transcriptional gene expression, referred to as miRNA, can be found in biofluids outside of the brain and can be associated with neurodegenerative diseases (Burgos et al, 2014; Jin et al, 2013). Recent studies have identified dysregulated miRNAs in cerebral spinal fluid and blood serum data from AD patients that may be useful in addressing disease progression (Burgos et al, 2014). Other studies have confirmed that saliva can be used to identify miRNA profiles in disease pathologies (Bonne and Wong, 2012; Park et al 2009). However, the miRNA profiles correlated with AD have yet to be examined via saliva. If in fact, dysregulated miRNAs can be found in saliva, similar to blood serum and CSF, this could lead to the development of a non-invasive procedure with the potential to explore the neuropathology directly associated with AD and a quantitative diagnosis.

In addition to dysregulated miRNA that has been observed in AD, olfactory deficits have been noted. For AD a deficit in smell has not only been associated with cognitive impairment, but has been used as a model to predict cognitive decline (Devanand et al, 2015). In this study it is hypothesized that subjects with AD will demonstrate a difference in the miRNA expression profiles in saliva and a decreased sense of smell compared to similar age matched controls.

METHODS

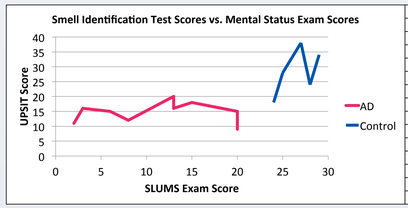
From the Clinic

- Patients were recruited by a geriatrician at the family medicine clinic at PCOM
- An inclusion/ exclusion criteria was used to determine eligibility of a subject (Figure 1)
- The St. Louis University Mental Status (SLUMS) Exam was used to determine a subjects cognitive ability (Figure 2: see bottom for scoring key)
  - Normal = Control Subject
  - Dementia = AD Subject
- The University of Pennsylvania Smell Identification Test (UPSIT) was used to screen for any potential olfactory deficits in subjects (Figure 3)
  - 40 scratch and sniff odorants each of which is force- choice
  - > 65 yo: Anosmia ≤18; Severe Microsmia 19 - 25
- Saliva was collected from subjects via passive drool (Salimetrics)
- Saliva was treated with protectant (Qiagen) for RNA stabilization and long-term storage

To the Lab

- miRNA extraction with Spike In-Control
  - Spike In-Control used to determine the reliability of the extraction
- cDNA for samples and a standard curve transcribed (Qiagen miScript II RT Kit) and a RT-PCR was done to determine recovery of Spike-In Control
- cDNA from samples used to run a pre-loaded Human Inflammatory Response & Autoimmunity Qiagen miScript miRNA PCR Array using the StepOnePlus™ Real-Time PCR System (Applied Biosystems™ ThermoFisher Scientific)
- The miScript Primer Assay for C. elegans miR-39 detects the miRNeasy Serum/Plasma Spike-In Control and this is used as a control (Figure 5: H1and H2 in array)
- Qiagen analytical program was used to analyze data (generate clustergrams and heat maps) from miRNA array to determine expression results from AD subjects in comparison to controls; quantification using the ΔΔCT method of relative quantification.

RESULTS



Subject	SLUMS Score	UPSIT Score
AD	2	11
AD	3	16
AD	6	15
AD	8	12
AD	13	20
AD	13	16
AD	15	18
AD	20	15
AD	20	9
Control	24	18
Control	25	28
Control	27	38
Control	28	24
Control	29	34

Figure 4: Line Graph and Chart showing UPSIT scores compared to SLUMS scores for 12 subjects. AD Subjects scored below 20 in both the UPSIT and SLUMS exam. Control Subjects scored above a 24 in the SLUMS exam and above 18 on the UPSIT.

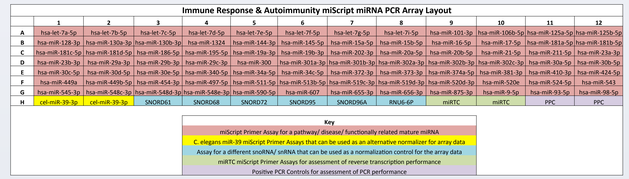


Figure 5: Chart showing the layout of the Human Inflammatory Response & Autoimmunity Qiagen miScript miRNA PCR Array and all of the miRNAs that are being analyzed and compared in AD and Control Subjects

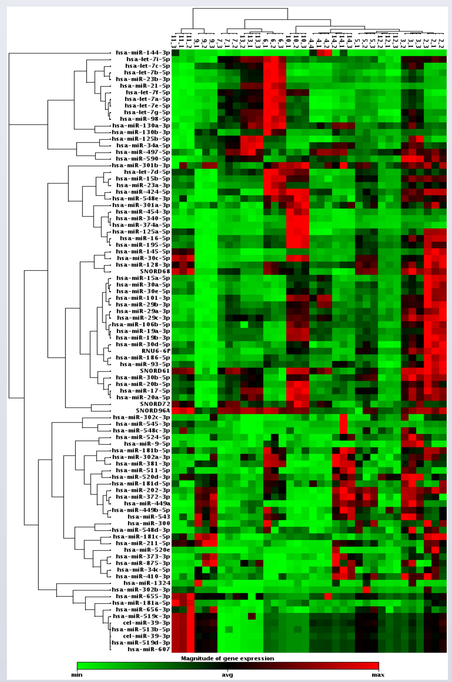


Figure 6: Clustergram created from the Human Inflammatory Response & Autoimmunity Qiagen miScript miRNA PCR Array of 12 patients in triplicate. The expression levels shown are determined by the C_T value.

A.	Age	Sex	SLUMS	Smell Test
Control	80	Female	29	34
AD	80	Female	2	11

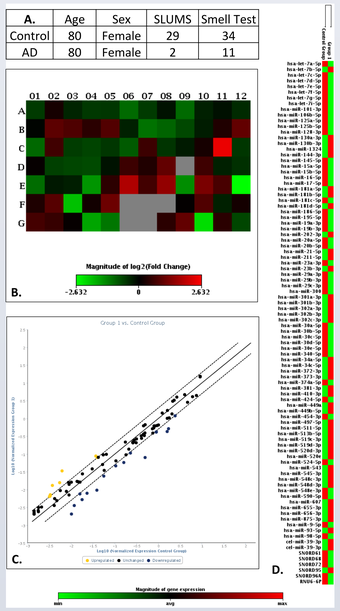


Figure 7: **80 year old Females (AD vs. Control)**
A: SLUMS and UPSIT Scores
B: Heat Map representing the fold change in each miRNA of the AD subject when normalized to the control
C: Scatterplot showing the number of miRNAs that are over and under expressed in the AD subject (Group 1) when normalized to the control
D: Clustergram demonstrating the gene expression levels for each individual miRNA calculated by the C_T value (Group 1 = AD Subject)

A.	Age	Sex	SLUMS	Smell Test
Control	90	Female	24	18
AD	90	Female	8	12

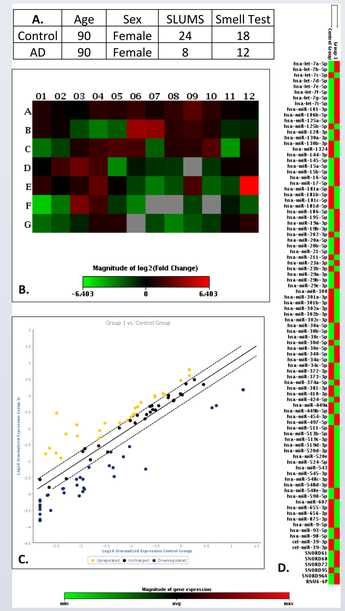


Figure 8: **90 year old Females (AD vs. Control)**
A: SLUMS and UPSIT Scores
B: Heat Map representing the fold change in each miRNA of the AD subject when normalized to the control
C: Scatterplot showing the number of miRNAs that are over and under expressed in the AD subject (Group 1) when normalized to the control
D: Clustergram demonstrating the gene expression levels for each individual miRNA calculated by the C_T value (Group 1 = AD Subject)

METHODS (CONT.)

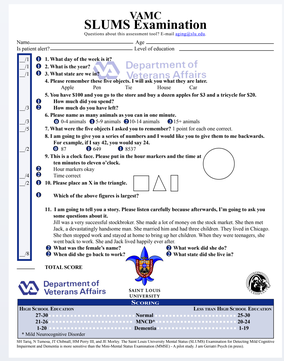


Figure 2: SLUMS Exam

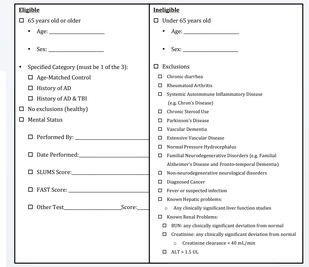


Figure 1: Inclusion/ Exclusion Criteria for Study Subjects

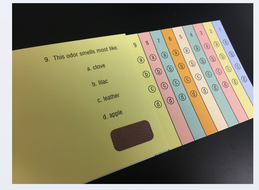


Figure 3: Sample Page of UPSIT

CONCLUSIONS

This pilot study is designed to evaluate the cognitive status and biomarkers such as olfactory testing and miRNA from saliva in order to determine if these components of evaluation could be used to better predict an individual with AD from an age and gender matched control individual.

- Preliminary data indicate a correlation between the UPSIT and SLUMS exam
- Initial analysis shows a change in miRNA regulation between AD when compared to it's control group
- Variability of miRNA expression levels between different age groups has been noted
  - Therefore, the number of subjects in each age group needs to be increased to determine levels of variability between ages in miRNA expression

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